Bacterial Lipopolysaccharide as Adjuvants

33

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Contents

Abstract

Lipopolysaccharide (LPS, endotoxin) is a major component of the outer membrane of gram negative bacteria. It is an activator of humoral and cellular responses in humans with potential use as adjuvant in vaccine technology. Importantly, LPS has a large capacity to induce Th1-type responses and stimulate cytotoxic T lymphocytes, which are poorly obtained by standard adjuvants but required for specific immune stimulatory therapies. In contrast, LPS possess an extreme toxicity that limit its clinical use in humans. Alteration of its chemical structure led the generation of LPS-based derivatives with reduced toxicity but retaining adjuvant properties. Monophosphoryl lipid A (MPLA) has been the most successful LPS-based adjuvant, currently incorporated in approved vaccine preparations and extensively used in vaccine trials and preclinical studies. Novel designed structures, analogous to LPS and generated by chemical synthesis, can offer lower production cost and lesser heterogenic formulations than MPLA and, in addition, be even most suitable for specific immune therapies. Thus, LPS-based structures are valuable contributions as adjuvants in human vaccinology and open new possibilities to existing demands for specific therapies.

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The development of subunit vaccines has improved the safety of human vaccine prophylaxis but requires strong adjuvants. Alum (diverse aluminum salts) is the most used adjuvant with an acceptable profile of side effects and induction of optimal protection against many human pathogens. However, alum is not suitable against certain pathogens or for new vaccine therapies like cancer or allergy. Lipopolysaccharide is a component of the outer membrane of gram-negative bacteria largely studied as adjuvants by their inherent ability to stimulate immune responses. In contrast, LPS induces inacceptable toxic effects in humans.

The generation of new detoxified LPS species with higher adjuvant characteristics than alum and acceptable toxicity has opened new perspectives to the vaccination. In this book chapter, the basic aspects of LPS structure, toxicity, and activation of the immune system are first introduced. Next, adjuvant characteristics of alum and corresponding drawbacks are briefly cited. Followed, the most promising detoxified LPS molecules and adjuvant characteristics are further discussed with special emphasis in current advances. A final overview section summarized the most relevant points.

33.1 LPS Structure and Biological Activity

Lipopolysaccharide is a component of the external leaflet of the outer membrane of gram-negative bacteria. It is a complex glycolipid formed by three domains, a fatty acid-rich domain (lipid A), an oligosaccharide domain (core), and a repeating oligosaccharide domain (O-antigen) [1]. Figure 33.1 represents the typical LPS organization.

The lipid A domain is a β-1, 6-linked D-glucosamine disaccharide linked to variable number of ester- and amide-linked 3-hydroxy fatty acids and phosphate groups (Fig. 33.1). Its architecture is highly conserved, but different microorganisms may present variations in the number and length of the fatty acid side chains, the presence of terminal phosphate residues, and associated

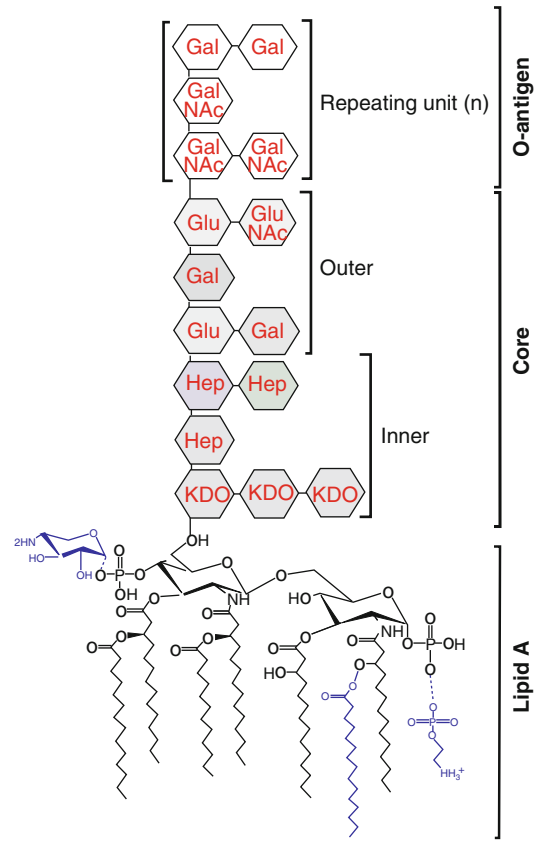

Fig. 33.1 General chemical structure of LPS of gram-negative bacteria. The LPS structure of *Escherichia coli* is depicted. LPS consists of three regions: lipid A, core, and O-antigen. The chemical structure of the lipid A of *E. coli* that displays the maximal immunostimulatory or endotoxic activities in humans (the topic of this work) is further detailed (*black colored*). Additionally, those substituents that possess the lipid A of *Salmonella minnesota* used to obtain MPL are blue colored. Residues of core and O-antigen region are schematized and abbreviated as *KDO* 2-keto-3-deoxyoctonoic acid, *Hep* D-GLYCERO-D-manno-heptose, *Glu* D-glucose, *Gal* D-galactose, *GluNAc* N-acetyl-glucosamine, and *GalNAc* N-acetyl-galactosamine. The length of the O-antigen depends on the number of repeating units. Additional substituents or modifications can be found in nature, but they are not shown for clarity

modifications. The core domain is a branched oligosaccharide region formed by nine or ten sugars, and its composition is more variable between species than lipid A. Finally, the O-antigen, if present, is the most variable of the tree domains and consists of up to 50 repeating oligosaccharide units formed of 2–8 monosaccharide moieties.

Table 33.1 Relevant effects of LPS in humans

Beneficial		Harmful	
Effect	Mechanism	Effect	Mechanism
Antimicrobial immunity	Activation of DC, priming B and T cells, opsonization, stimulation of natural killer cells, activation of macrophages	High fever	IL-1 release
Antiviral immunity	Stimulating CD8(+) T-cell immunity	Strong inflammatory responses	Large secretion of various inflammatory mediators
Antitumor immunity	Stimulating CD8(+) T-cell immunity	Disseminated intravascular coagulation	Reduction of elements involved in blood coagulation
Mitigation of Th2 response to Th1	Enhancing Th1 phenotype	Shock, hypotension, lymphopenia	Reduced blood flow

In addition, certain modifying enzymes can alter the composition of LPS contributing to increase the LPS heterogeneity [2–5].

Toll-like receptors (TLR)s belong to a family of receptors that recognize a broad diversity of specific but conserved structures of pathogen microorganisms [6]. Immediately after stimulation, TLRs initiated the activation of immune defense mechanisms. Toll-like receptor 4 (TLR4), in complex with the glycoprotein MD-2, constitutes the LPS receptor [7, 8]. TLR4 is a membrane-spanning protein present on antigen-presenting cells (APC) (macrophages and dendritic cells) and epithelial cells of humans. Its stimulation requires the cooperation of associated molecules, like the LPS-binding protein and CD14 that facilitate LPS transfer to the receptor. TLR4 stimulation induces the formation of intracellular protein complexes that leads to the activation of intracellular signaling cascades [9, 10]. These reactions trigger the biosynthesis and secretion of diverse proinflammatory cytokines (IL-1, IL-8, IL-12, TNFα, and IFNγ) and the production of co-stimulatory molecules [11] that finally activates humoral and cellular responses including activation of the complement system [12, 13], activation of macrophages [14, 15] B and T cells, and enhancement of cellular cooperation [11]. Consequently, this response is beneficial for the control of local infections. In contrast, high LPS dose, specially released to the blood system during sepsis, leads to large secretion of cytokines and inflammatory mediators with severe [16, 17] and/or fatal consequences [18, 19]. Table 33.1 summarizes the beneficial and harmful effects of LPS in humans, but for further details see revision [20, 21]. In summary, LPS is a strong activator of the immune system (adjuvant) but also a highly toxic substance (endotoxin).

The lipid A region is the major responsible of the TLR4 stimulation. Variations in its structure, mainly regarding the number and length of acyl acid chains, and the charge are crucial in this regard [22]. The hexa-acylated *E. coli* lipid A (canonical LPS structure and depicted in Fig. 33.1) with fatty acids of 12–14 carbons and two phosphate residues is the maximal stimulator of human TLR4 (hTLR4) [23, 24]. In contrast, the tetra-acylated lipid IVa with fatty acids of 18–16 carbons and a phosphate residue, an intermediate in the biosynthetic pathway of lipid A, does not stimulate hTLR4 (canonical hTLR4 antagonist) [25].

33.2 Lipid A Analogous Structures and Its Role as Adjuvants

Vaccine based on infectious attenuated or inactivated whole pathogens contains a large variety of target structures for TLRs and, subsequently, promotes strong and long protection. However, they generate a large variety of side effects even with fatal consequences [26, 27]. Vaccines based on one or certain purified components (subunit

vaccines) show acceptable safely but a poor immunogenicity and require additional immune stimulators (adjuvants). Alum refers to several aluminum salts and is the most used adjuvant. It is safe and elicits predominantly a Th2-type antibody response that shows to be effective in a large variety of vaccines [28]. However, alum hardly promotes Th1-type antibody responses [29]. Adjuvants that favor Th1 or more balanced Th1/Th2 responses are required to induce optimal immune protection against certain pathogens [30] or diseases as cancer [31] or allergy [32]. Apart from that, alum poorly stimulates mucosal immunity. Mucosa tissues are the first line of defense against many pathogens and the ecological niche of commensal and opportunistic microorganisms, for example, *Neisseria meningitidis*.

Therefore, mucosal immunity is considered the gold therapy to evade pathogen colonization and confer herd immunity against certain particular pathogens. Vaccine adjuvants that target mucosal immunization must promote a large series of biological and complex activities as Th17 cell development, APC proliferation, and IgA production [33, 34]. In this regard, several substances have been extensively studied as bacterial toxins or CpG, among others [34], but, till date, no available approved adjuvant exists (with the exception of MPL, to be discussed next). Alternatively to alum, three additional adjuvants were licensed: MF59, AS03, and RC-529. MF59 is an oil in water emulsion with low oil content, and it is included in an approved influenza vaccine [35]. Although MF59 induces a more balanced Th1/Th2 response than alum, it shows partial efficacy, often requiring the coadministration of Th1 enhancers. ASO3 and RC-529 contain LPS-based substances to be discussed later.

LPS has attracted large attention as adjuvant by its high capacity to induce Th1-type responses against coadministrated antigens. One of the most relevant factors involved in the development of this response is IL-12. Note that LPS is a stimulator of this interleukin. Interestingly, TLR4 receptors are tactically present at mucosa surfaces; therefore, it would be expected that TLR4 agonists can promote immune responses at local and distal mucosal sites. In the past decades, several strategies were followed to reduce its extreme toxicity without altering this inherent capacity; variation of the LPS composition, in particular lipid A, by chemical treatments and chemical synthesis of lipid A analogues is a good example (see detailed revision [36]). As a result, a diverse lipid A species was generated, although only few exhibited the desirable properties. Next, the most relevant substances and clinical applications are further discussed.

The chemical hydrolysis of the LPS of *S. minnesota,* which contains a lipid A with seven acyl chains and three phosphate groups as depicted in Fig. 33.1 (with blue-colored substituent), generated one of the most successful LPS-based adjuvants, the monophosphoryl lipid A (MPL). This derivate structure is a six-acyl side chain lipid A with one phosphoryl group [37] (see Fig. 33.2). MPL demonstrated to be less toxic than the parent (0.1% of toxicity) [38] with a toxic side effect profile comparable to alum [39, 40] while retaining the stimulatory properties of LPS. At present, MPL is adjuvant of approved vaccine preparations for humans in Europe (human papillomavirus (Cervarix) [33, 34] and pollen allergy (Pollinex Quattro) [41, 42]) and Australia (hepatitis B virus (Fendrix) [43]), and it has been used extensively in human vaccine trials for several infectious diseases like malaria [44], tuberculosis, [45, 46] or tumor growth [47].

Because MPL is highly hydrophobic and generates aggregates in aqueous solution that may considerably affect the TLR4 activation, it is often formulated in combination with alum or other delivery systems [48]. These combinations, together with other factors (accompanying antigen or administration route), can alter its adjuvant action. For example, in aqueous formulations MPL promotes antibody production, while in oil in water emulsions, it better stimulates T-cell responses. In contrast, MPL combined with other delivery systems is a strong stimulator of cytotoxic T lymphocyte proliferation. Delivery systems can also modify their biological properties. Liposomes are spherical vesicles formed by phospholipid bilayers extensively used to deliver antigens in its native conformation. Incorporation of MPL into liposomes reduced the residual

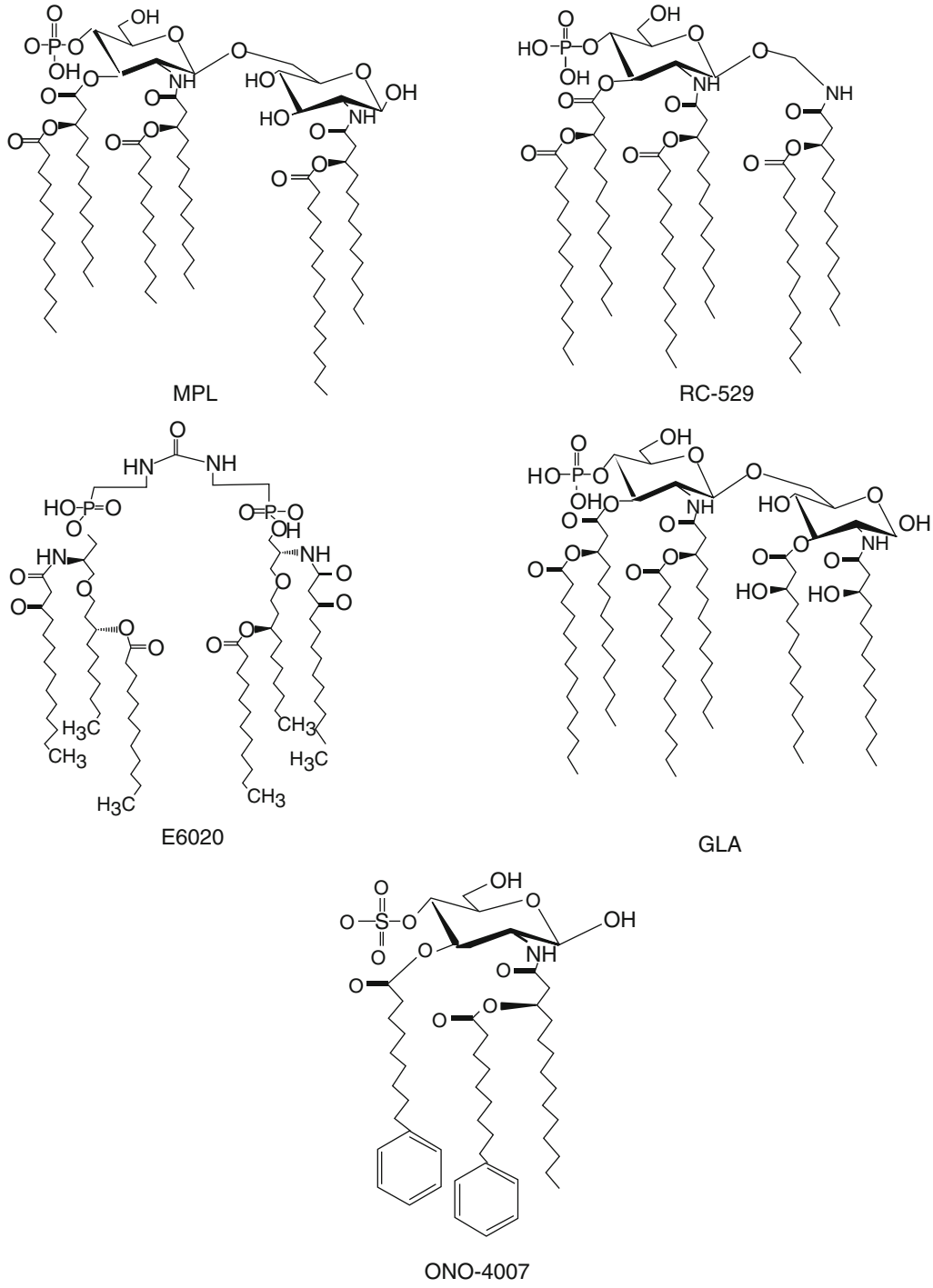

Fig. 33.2 Chemical structure of relevant LPS-based adjuvants

toxicity of MPL but retained intact its adjuvant potential [49]; this effect was also observed with other detoxified LPS species [50]. Therefore, liposomal MPL formulations were extensively studied in human trials for different indications as malaria [49], pneumococcal disease, [51] or

genital herpes type 2 [52] and in experimental animals for *Streptococcus pyogenes* infections [53] or toxin neutralization of *E. coli* [54]. Finally, it is significant to notice MPL's success in providing mucosal immunity after mucosal [55] and intramuscular [56, 57] administration; MPL formulations for mucosal vaccines have been extensively explored for the treatment of different diseases; genital herpes [58] and mucosal leishmaniasis [59] are good examples.

One of the main drawbacks attributed to MPL is the heterogeneity of lipid A congeners generated during its production with subsequent purification cost. A solution to this problem could be the generation of synthetic lipid A analogues. Chemical synthesis generates pure and defined structures that reduce production cost. Like natural LPS species, these structures interact with hTLR4. Therefore, numerous analogues with variation in acyl chain length and positions, phosphate groups, or the backbone unit were generated and their biological activity further analyzed. Till date, the most suitable molecules in vaccine development are RC-529, E6020, GLA, and ONO-4007 (see chemical structure in Fig. 33.2).

RC-529 is a synthetic analogue of MPL composed by a monosaccharide backbone with six fatty acyl chains [38]. It is a very attractive adjuvant. Like MPL, it is well tolerated and effective during clinical trials [38] but with a lower production cost. In fact, its use was approved in Argentina in a hepatitis B vaccine. It is often in combination with different delivery systems to enhance its solubility or improve its delivery without affecting immune stimulatory capacities [60]. In addition, several studies performed in experimental animals indicate that RC-529 is an efficient mucosal adjuvant against pathogens that lack effective vaccine therapy. For example, it elicited bactericidal antibodies after intranasal immunization with the *Streptococcus pneumoniae* protein PppA [61] and the meningogoccal protein P2086, [60] and it promoted high antibody titers in macaques at the nasal and genital mucosa against an HIV peptide immunogen [62]. Similarly, it reduced nasal colonization of nontypable *Haemophilus influenzae* and *Moraxella catarrhalis* in mice that were immunized via nasal with recombinant proteins, [63] and it conferred significant protection against lethal influenza challenge [64].

E6020 is an hexa-acylated acyclic backbone [65, 66] with higher biological activities than alum [65, 66] or MPL [65] and with a reduced toxicity [67]. Its simple structure allows the production of high-purity material than other synthetic TLR4 agonists [65]. Various works showed its high capacity to shift immune responses towards a Th1 profile when combined with conventional vaccines [65, 67, 68]. Generation of this immune profile is especially relevant in cancer vaccines. Indeed, E6020 in combination with a monoclonal antibody (trastuzumab) enhanced significantly protection against tumor growth in animal models [69].

GLA is a hexa-acyl synthetic lipid A derivative composed of a disaccharide backbone with a single phosphate group. Results show that it has even more powerful adjuvant abilities than MPL [70, 71], and it exhibited a good safety profile in animals and in Phase I trials [72]. MPL has a strong but not overwhelming ability to promote Th1 responses. Interestingly, GLA exhibits a strong ability to shift antigen-specific immune responses towards Th1 type [73, 74]; hence, it is being proposed as a better alternative to MPL to confer adequate protection against certain pathogens. In fact, significant protection in animals of experimentation was reported against *Toxoplasma gondii*, [75] *Mycobacterium tuberculosis*, [76, 77] or influenza virus [72].

ONO-4007 is a tri-acylated acyclic sulphonated backbone. This molecule induced tumor and metastases regression in animal models [78, 79]. This property was due to its strong ability to stimulate secretion of tumor necrosis factor (TNF-α) by macrophages [80, 81]. Studies in rodents showed remarkable but selective efficacy against TNF-α-sensitive tumors, which improved in combination with other antitumor therapies [82]. Unfortunately, only a primed state induction of TNF-α was detected in human cells [83]. Phase I clinical studies revealed a limited capacity and the antitumor studies were not continued [79]. In contrast, the molecule exhibits antileishmanial [84] and anti-allergy activities [85].

Conclusions

Although LPS was long known as an immune stimulatory substance with potential adjuvant use, the large variety of unacceptable toxic effects drastically restricted its clinical use. However, the finding that MPL was safe and retained the desirable adjuvant properties of LPS opened new possibilities to treat pathogen diseases. In contrast to previous adjuvants, LPS-based adjuvants offer new benefits from their ability to enhance Th1-type responses and stimulate cytotoxic T lymphocytes. This activity is essential to confer protection against many pathogens and to develop prophylaxis therapies against other diseases as cancer or allergy. Indeed, this is supported by the efficacy of MPL in available vaccines whereas standard adjuvants failed to provide protection. Additionally, the high adjuvant capacity of LPS-based adjuvants has considerable and obvious benefits in mucosa protection, faster activation of protection, reduction of booster doses, functional immunization in elderly, or preparation of polyvalent vaccine formulations. Certain drawbacks were attributed to MPL, e.g., elevate production cost and possible activation/enhancement of TLR4-related autoimmune diseases. Synthetic lipid A analogues with similar biological activity like MPL have demonstrated considerable reduction of the production cost. In regard to the activation TLR4 autoimmune diseases, accumulated data till date from immunization in humans provides further evidence of safety. In summary, LPS-based adjuvants improve the current vaccination therapies and open possibilities to solve their existing challenges.

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